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Activation of Liver Disease in Healthy Hepatitis B Surface Antigen Carriers During Interferon-Alpha Treatment

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Fifty percent of healthy hepatitis B surface antigen carriers may have histologically proven chronic hepatitis. Our aim was to study the benefit of interferon-alpha in healthy patients. Twenty-nine hepatitis B surface antigen carriers with normal liver enzymes and with serum hepatitis B virus DNA were randomized into two groups: Group I, 14 patients treated with 9 megaunits of interferon alpha-2a thrice weekly for six months, and Group II, 15 control patients. A liver biopsy was obtained from each patient at study initiation. A second biopsy was available in nine treated patients and six controls. During treatment, a significant increase in alanine amino transferase levels was observed in treated patients as compared with the controls ($P < 0.05$). After treatment, transaminase levels decreased to normal values. No differences between treated and control patients were observed in clearance of hepatitis B virus markers. A significant increase in the total histological activity index between base line and final liver biopsies was observed in treated patients ($P < 0.05$). It is concluded that interferon alpha treatment may induce a biochemical and histological activation of liver disease. Accordingly, interferon alpha should not be administered to healthy hepatitis B surface antigen carriers, at least with the schedule used in this work. ***J. Med. Virol. 53:76–80, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: hepatitis B virus; healthy carriers; interferon treatment

INTRODUCTION

Hepatitis B virus (HBV) infection is a worldwide problem, with approximately 350 million carriers. During the natural history of chronic hepatitis B infection, two phases may be recognized. In the first years of the infection, high replication levels of HBV are detected, with serum hepatitis B e antigen (HBeAg) and HBV DNA and a progressive liver disease. Either spontaneously, in about 25% of patients per year [Realdi et al., 1980; Hoofnagle et al., 1981], or after successful interferon (IFN) alpha treatment (40% of patients treated) [Hoofnagle et al., 1988; Carreño et al., 1994], seroconversion to antibody to hepatitis B e antigen (anti-HBe) with clearance of HBV replication markers may occur and the disease becomes inactive, with alanine aminotransferase (ALT) normalization and improvement in the liver histology. Those patients with hepatitis B surface antigen (HBsAg), anti-HBe and normal ALT may be classified as healthy carriers; however, healthy carriers may have serum or liver HBV DNA. Furthermore, up to 50 % of healthy HBsAg carriers with constantly normal ALT may have histologically proven chronic hepatitis [De Franchis et al., 1980].

No therapeutic attempt has been made to clear HBV DNA and improve the possible histological lesion in healthy carriers.

The aim of our study was to determine the possible benefit (virological and histological) of IFN alpha for the treatment of healthy HBsAg carriers as compared with untreated carriers.

MATERIALS AND METHODS

The sample size was calculated by a computer, using the EPISTAT program. Considering a possible drop-out rate of about 20%, and assuming no loss of HBV DNA in control patients and a 40% HBV DNA clearance rate in treated patients, a minimum of 14 patients and 14 controls were required to detect a significant difference at a 95% confidence interval with a power of 80%. Accordingly, in the study were included 29 con-

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TABLE I. Base Line Features of Patients

	Treated (n = 14)	Controls (n = 15)	*P*
Age (years)[a]	39.6 ± 12.0	39.4 ± 11.5	n.s.
Sex (M/F)	10/4	6/9	n.s.
ALT (IU/L)[a]	31.6 ± 13.0	25.7 ± 7.8	n.s.
Time with normal ALT levels (months)[a,b]	49.0 ± 38.7	82.8 ± 51.1	n.s.
Histology			
Minimal chronic hepatitis			
No fibrosis	9	8	n.s.
Mild fibrosis	2	5	n.s.
Moderate fibrosis	1	—	n.s.
Severe fibrosis	—	1	n.s.
Mild chronic hepatitis			
No fibrosis	2	1	n.s.
Histological activity index[a]	2.5 ± 0.9	3.2 ± 1.1	n.s.

[a]Mean value ± SD.
[b]Time elapsed since the discovery of serum HBsAg in each case.

secutive healthy HBsAg and anti-HBe positive patients without HBV DNA detectable by dot-blot hybridization with constantly normal ALT levels (upper normal limit: 45 IU/L), as checked every six months after the diagnosis, for a mean period of 67.38 ± 51.76 months. For inclusion in the study, the patients also had to have serum and liver HBV DNA detectable by polymerase chain reaction (PCR), as well as histologically proven damage according to Desmet et al. [1994] (Table I) in a liver biopsy obtained within six months of entry to the study. The patients included had never been treated with an antiviral or immunosuppressive therapy. The base line characteristics of the patients are summarized in Table I. Clinical and biochemical analyses were performed monthly during therapy and the 6 months of post-treatment follow-up. A second liver biopsy was obtained from 15 patients (nine treated and six controls) from 7 to 14 months after therapy (mean: 11.3 ± 2.6 months in treated patients and 9.2 ± 4.1 months in controls). Histological activity index in the first and final liver biopsies was assessed according to Knodell et al. [1981].

The patients were randomized into two groups: Group I, 14 patients treated with 9 megaunits of recombinant IFN alpha-2a (Roferon A, Hoffmann-La Roche, Basel, Switzerland) three times weekly for six months, and Group II, 15 untreated patients who served as controls and were studied under the same conditions as the patients treated. All patients gave written informed consent, and the trial was approved by the ethics committee of the hospital.

HBsAg, HBeAg and anti-HBe were tested by commercial radioimmunoassay (Abbott Laboratories, North Chicago, IL). Anti-IFN antibodies were tested by enzyme immunoassay (Anawa Biochemical, Zürich, Switzerland). Liver function tests were analyzed by standard methods (Smac 20, Technicon, New York, NY).

Viral DNA from serum samples was isolated as described elsewhere [López-Alcorocho et al., 1994]. Liver HBV DNA was isolated using the method described previously [Bartolomé et al., 1990]. The total DNA concentration isolated from the liver biopsies was determined measuring its absorbance at 260 nm. The pre-core region of the HBV genome was amplified by PCR in a final volume of 50 μL containing 10 μL of serum DNA or 1 μg of liver DNA samples, 10 mmol/L Tris-HCl pH 8.3, 50 mmol/L KCl, 2 mmol/L $MgCl_2$, 0.1% Triton X-100, 200 μmol/L dNTPs, 0.5 Units of Taq DNA polymerase (Promega Co., Madison, WI) and 0.2 μmol/L of each primer M2 (nucl. pos. 2370–2394) and M3 (nucl. pos. 1730–1754), for 30 cycles (94°C, 1 min; 55°C, 1 min; 72°C, 1.5 min). A second round of PCR was carried out with 10 μL of the first PCR product, using primers B1 (nucl. pos. 1776–1804) and BC1 (nucl. pos. 1955–1974) for 30 cycles (94°C, 1 min; 55°C, 1 min; 72°C, 1.5 min). The nucleotide position of the primers is stated according to Ono et al. [1983].

In order to avoid false positive results, the Kwok and Higuchi [1989] contamination-prevention measures were followed. As negative controls, three serum samples from healthy donors without HBV markers were included in each PCR assay. All serum samples were tested in duplicate and analyzed in a blinded fashion.

The predominant HBV pre-core variant, with a single change from G to A at position 1896, was determined in the base line and final samples by a specific oligonucleotide hybridization assay of nested PCR products, using probes for the wild-type HBV and pre-core mutant (G to A substitution at position 1896), as described previously [López-Alcorocho et al., 1994].

The Chi-square and Fisher's exact tests were used for comparison of proportions. Continuous variables were analyzed by Student's *t*-test.

RESULTS

The base line characteristics were similar in both groups (Table I). Surprisingly, after one month of treatment, a significant increase in ALT was observed in the treated patients, while no changes were found in the controls (treated vs. controls, 51.92 ± 31.39 vs. 25.13 ± 8.20 IU/L: $P < 0.01$) (Fig. 1). This trend remained the same during the entire treatment period, and the ALT values were always statistically higher than the base line levels (with the exception of the last serum sample of treatment). In contrast, no changes in the ALT levels were observed in the controls during these 6 months, and the differences with respect to those of the treated patients were always significant (Fig. 1).

After three months of IFN alpha therapy, 6/14 treated patients (43%) had abnormal ALT levels, but this occurred in none of the controls ($P < 0.05$) (Fig. 2). In the final sample treatment, 5/14 patients from the treated group (36%) had abnormal ALT levels, while all the controls continued to have normal ALT values ($P < 0.05$). After treatment end, the ALT levels decreased progressively, and, at twelve months of follow-up, all treated patients, as well as the controls, had normal ALT (Fig. 2).

With respect to serum HBV DNA, one of the treated

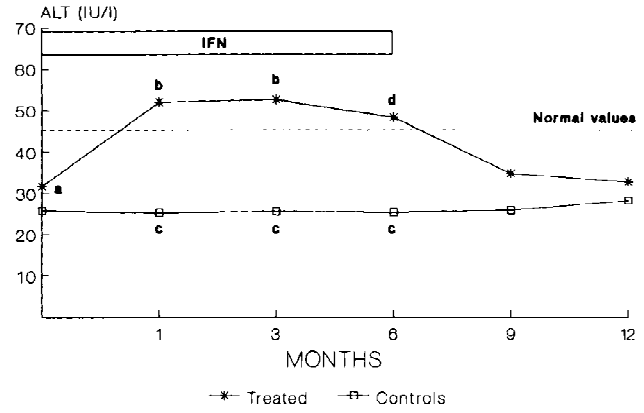

Fig. 1. Changes in ALT levels during treatment and follow-up periods. a vs. b: $P < 0.01$; b vs. c: $P < 0.01$; d vs. c: $P < 0.05$ (upper normal limit of ALT: 45 IU/L).

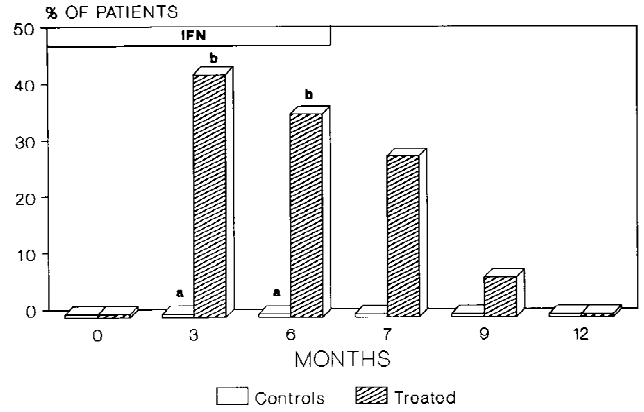

Fig. 2. Percentage of patients with abnormal ALT levels during treatment and follow-up. a vs. b: $P < 0.05$.

patients (7%) became negative at the end of follow-up, while another lost serum HBV DNA at the end of treatment, with reappearance at the end of follow-up. None of the controls cleared serum HBV DNA. Serum HBsAg became undetectable in 3/14 (21%) treated patients and 2/15 (13%) controls without significant differences between the groups. No correlation between HBV DNA, or HBsAg clearance, and liver histology or known duration of the HBsAg carriage was observed (data not shown).

A second liver biopsy was obtained from nine treated patients and six controls. When comparing the total histological activity index of the base line and final biopsies, a significant increase was observed in the treated patients (base line 2.50 ± 0.98 vs. final 3.80 ± 2.00; $P < 0.05$) but not in the controls (base line 3.20 ± 1.10 vs. final 3.83 ± 1.34; n.s.). This increase was at expense of periportal necrosis, portal inflammation and fibrosis although the increase of each individual score did not reach statistical significance (Table II). No relation was found between the time of the second liver biopsy and deterioration of liver.

With respect to the HBV pre-core genotypes, in the base line sample, 34% of the patients had wild-type HBV and 48% a mixture of wild-type and pre-core mutant; in five cases no serum was available for HBV genotyping. When comparing the HBV genotype distribution between treated patients with or without an ALT increase during therapy, no significant differences were observed (ALT increase: wild-type, 33%; mixture, 67%; no ALT increase: mixture, 100%). No substantial changes in HBV genotype distribution were observed when comparing the base line and final serum samples (this latter taken at the treatment end) in either treated or control patients (Table III).

The secondary effects were as described previously with IFN [Quesada et al., 1984]. Four treated patients developed anti-IFN antibodies (three of these had an ALT increase during treatment). One of these four patients cleared HBsAg. In addition, there was no relation between the appearance of anti-IFN antibodies and liver histology.

DISCUSSION

Healthy HBsAg carriers with anti-HBe have a generally good prognosis without developing liver disease [Lok and Lai, 1988]. However, as many as 50% of these patients may have histologically proven liver disease even in the presence of constantly normal ALT levels [De Franchis et al., 1980]. Furthermore, reactivation of HBV in healthy carriers with an increase in ALT and the development of active chronic hepatitis have been reported [Davis et al., 1984; Castillo et al., 1990]. In addition, between 50 and 70% of HBsAg healthy carriers may have serum HBV-DNA as detected by PCR [Chemin et al., 1991; Luo et al., 1992]. For all these reasons, a controlled study of IFN alpha treatment was undertaken in healthy HBsAg, anti-HBe carriers with normal ALT, HBV DNA in serum and liver, and histologically proven liver damage.

After 1 month of treatment, a significant increase above the upper limits of normality in ALT took place in treated patients and after three months as many as 43% of these patients had abnormal ALT levels. However, all the untreated controls continued to have normal ALT during the study, with significant differences between both groups. This result demonstrates that IFN alpha treatment of HBsAg healthy carriers induces a biochemical activation of the liver disease. An explanation for this finding is not clear but several possibilities may be considered. The presence of pre-core HBV mutants has been associated with acute fulminant hepatitis [Yoshiba et al., 1992; Uchida et al., 1993] and with severe chronic hepatitis [Omata et al., 1991]. However, pre-core mutants were found in 48% of the healthy HBsAg carriers with minimal liver damage and normal ALT. This finding is in agreement with previous reports [Tur-Kaspa et al., 1992] and would suggest that the presence of pre-core mutants alone is not necessarily associated with aggressive liver disease. On the other hand, ALT increase during the IFN alpha therapy may be due to the appearance of HBV escape mutants under the immunological pressure of IFN alpha, as has been described previously [Günther

TABLE II. Scoring of Histological Activity Index Components in the Base Line and Final Liver Samples in Treated and Control Patients

	Treated (n = 14)		Controls (n = 15)	
	Base line	Final	Base line	Final
Periportal necrosis	0.20 ± 0.44	0.78 ± 1.64	0.33 ± 0.52	0.17 ± 0.41
Intralobular degeneration	1.22 ± 0.83	1.11 ± 0.78	1.00 ± 0.00	1.00 ± 0.00
Portal inflammation	0.44 ± 0.53	0.78 ± 0.97	1.17 ± 0.98	1.50 ± 1.25
Fibrosis	0.55 ± 0.73	0.89 ± 1.17	0.83 ± 0.41	1.17 ± 0.41
Total	2.50 ± 0.98[a]	3.80 ± 2.00[b]	3.20 ± 1.10	3.83 ± 1.34

[a] vs. [b] $P < 0.05$.

TABLE III. Changes in HBV Pre-core Genotypes in Treated and Control Patients

	Treated (n = 14)		Controls (n = 15)	
	Base line	Final	Base line	Final
Wild-type	3 (21%)	2 (14%)	7 (46%)	—
Mixture	10 (72%)	10 (72%)	4 (27%)	15 (100%)
Not done	1 (7%)	2 (14%)	4 (27%)	—

et al., 1992]. We did not find differences at least in the pre-core region when comparing the base line and final samples; thus, escape mutants in this region of the HBV genome do not seem to play a role in the ALT increase observed in our patients. However, the association of mutations in other regions of the viral genome with disease activity cannot be discarded. Another possibility is that IFN alpha induces an immune stimulation with a better expression of the HLA-Class I molecules and recognition by T lymphocytes with clearance of HBV [Pignatelli et al., 1986]. However, this hypothesis appears unlikely because, were this the case, HBV clearance would be achieved. In this context no significant differences in the frequency of HBsAg negativization or serum HBV DNA clearance were observed between treated and control healthy HBsAg carriers. Finally, several investigators [Real et al., 1986; Kabbash et al., 1988; Leventhal et al., 1988] found that IFN alpha treatment may lead to liver toxicity, with an ALT increase in different diseases. In this sense, it has been reported that IFN alpha may increase ALT levels in as many as 64% of patients with recurrent respiratory papillomatosis who had normal base line ALT values and were treated with a similar dose and type of IFN alpha as were used in our study, and for the same treatment period [Leventhal et al., 1988]. Moreover, IFN alpha has been used to treat the AIDS-associated Karposi's sarcoma, and a direct relation between ALT increase and IFN treatment was reported in these patients [Real et al., 1986; Kabbash et al., 1988]. Accordingly, direct toxicity of IFN alpha in the liver may also explain the increase in ALT which we observed in HBsAg healthy carriers with a normal base line level of ALT. In favour of this hypothesis is the fact that the increase in ALT levels was transitory, all patients having normal ALT six months after IFN alpha treatment.

With respect to liver histology, a deterioration was found when comparing the base line and final liver biopsies of treated patients, while no significant changes were observed in the liver biopsies of controls. The increase in histological activity was at the expense of periportal necrosis portal, inflammation and fibrosis. Therefore, the histological damage induced during IFN alpha treatment of HBsAg healthy carriers is not transitory, lasting for at least six months after treatment end. However, we cannot discard a regression in the liver histology thereafter, as occurred with the ALT increase.

For the above reasons, IFN alpha treatment should not be administered, at least with the schedule used in this work, to HBsAg healthy carriers with normal ALT, irrespective of the presence of a chronic hepatitis or serum HBV DNA. In the future, other drugs with specific antiviral effects and a lack of hepatotoxicity such as lamivudine [Schalm et al., 1995] may be attempted in these patients.

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